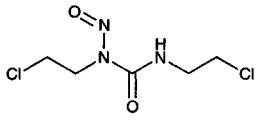
Carmustine



Molecular formula: $C_5H_9Cl_2N_3O_2$
Molecular weight: 214.05
CAS Registry No.: 154-93-8
Merck Index: 1894
Lednicer No.: 2 12

SAMPLE

Matrix: blood
Sample preparation: 2-5 mL Whole blood or plasma + 32 mg sodium ascorbate + 200 mg phenytoin + 2-5 mL MeCN, shake or vortex for about 5 min, centrifuge at 2000 rpm for 5-10 min, filter (Millipore sample filtration kit), inject an aliquot of the filtrate

HPLC VARIABLES

Guard column: Altex
Column: 150 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeOH:water 50:50
Flow rate: 1
Detector: UV 237

CHROMATOGRAM

Retention time: 6
Internal standard: phenytoin (9)
Limit of detection: 100 ng/mL

KEY WORDS

whole blood; plasma; handle under yellow light

REFERENCE

Krull,I.S.; Strauss,J.; Hochberg,F.; Zervas,N.T. An improved trace analysis for N-nitrosoureas from biological media, *J.Anal.Toxicol.*, **1981**, *5*, 42–46.

SAMPLE

Matrix: blood
Sample preparation: Acidify plasma by adding 4% (v/v) glacial acetic acid to a final pH of 4. 500 μL Plasma + 1.68 μg propyl paraben in EtOH:water 5:95, vortex for 5 s, add 4 mL diethyl ether:EtOH 98.75:1.25, shake mechanically for 15 min, centrifuge at 2000 g for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of air at 35°, reconstitute the residue in mobile phase, vortex for 10 s, inject an aliquot

HPLC VARIABLES

Guard column: 70 × 2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:0.1% acetic acid 35:65
Flow rate: 1.2
Detector: UV 230

CHROMATOGRAM

Retention time: 13.8
Internal standard: propyl paraben (16.9)
Limit of detection: 50 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE
Yeager,R.L.; Oldfield,E.H.; Chatterji,D.C. Quantitation of 1,3-bis(2-chloroethyl)-1-nitrosourea in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *305*, 496–501.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg CBA Bond Elut SPE cartridge with 1 mL MeOH and 1 mL water. Centrifuge blood at 5000 g for 2-3 min, freeze plasma in dry ice/hexane within 1 min. Thaw within 3 min by immersion in a 50° water bath. 1 mL Thawed plasma + 500 μL 2.5 μg/mL IS in 100 mM citric acid, vortex for 5 s, centrifuge for 5 min, add a 1 mL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, elute with 200 μL MeOH into a vial containing 50 μL 100 mM acetic acid, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 125 × 5 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM ammonium acetate 30:70 adjusted to pH 4.4 with glacial acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 8.5
Internal standard: 1-methyl-3-isobutyl-8-vinyl-2,6-dioxopurine (S10338) (7.2)

OTHER SUBSTANCES
Extracted: lomustine, fotemustine

KEY WORDS
plasma; SPE

REFERENCE
Gordon,B.H.; Richards,R.P.; Hiley,M.P.; Gray,A.J.; Ings,R.M.; Campbell,D.B. A new method for the measurement of nitrosoureas in plasma: an h.p.l.c. procedure for the measurement of fotemustine kinetics, *Xenobiotica*, **1989**, *19*, 329–339.

SAMPLE
Matrix: blood
Sample preparation: Add fotemustine in ethanol PBS to plasma. Mix 100 μL plasma with 600 μL diethyl ether, rotate, centrifuge. Remove 400 μL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 mm long 4 μm Novapack
Mobile phase: EtOH:1% acetic acid (pH 3) 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.6
Internal standard: fotemustine (7.6)
Limit of detection: 1000 ng/mL

KEY WORDS
plasma; rat

REFERENCE

Meulemans,A.; Giroux,B.; Hannoun,P.; Henzel,D.; Bizzari,J.P.; Mohler,J. Permeability of two nitrosoureas, carmustine and fotemustine in rat cortex, *Chemotherapy*, **1989**, *35*, 313–319.

SAMPLE

Matrix: reaction mixtures

Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ (pH 4.7) 55:45
Flow rate: 1
Injection volume: 20
Detector: UV 228

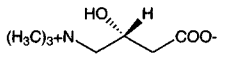
CHROMATOGRAM

Retention time: 8.0
Limit of detection: 160 ng/mL

REFERENCE

Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

Carnitine



Molecular formula: $C_7H_{15}NO_3$
Molecular weight: 161.20
CAS Registry No.: 461-06-3, 541-15-1 (L-form)
Merck Index: 1898

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg LiChrolut SCX SPE cartridge (Merck) with 2 mL water. Condition a Toyopak IC-SP S SPE cartridge (Tosoh) with 2 mL water. 50 μL Plasma + 10 μL 400 μM IS in water + 1 mL 25 mM pH 1.0 sodium phosphate buffer, mix, add to the LiChrolut SCX SPE cartridge, wash with 5 mL water, elute with 1 mL isopropanol:150 mM pyridine in water 50:50. Evaporate the eluate under reduced pressure, add 20 μL 2.5 mM 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole in DMF, add 20 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in DMF, add 10 μL pyridine, vortex, let stand at room temperature for 1 h, add 1 mL DMF:water 40:60, add to the Toyopak IC-SP S SPE cartridge, wash with 1 mL DMF:water 40:60, wash with 10 mL water, wash with 100 μL 600 mM KCl in MeOH:water 50:50, elute with 200 μL 600 mM KCl in MeOH:water 50:50, inject an aliquot of the eluate. (Synthesis of the reagent, 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole, is as follows. Add 3.15 g benzil and 2.46 g methyl 4-formylbenzoate (terephthalaldehydic acid methyl ester) to 10 g ammonium acetate in 30 mL acetic acid, stir at 80° for 9 h, cool to room temperature, pour into cold water, filter. Wash the precipitate with water and recrystallize it from EtOH to give 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoic acid methyl ester as pale yellow crystals (mp 245-248°). Reflux 1.47 g 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoic acid methyl ester and 15 mL 80% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) in 100 mL EtOH for 4 h, cool, to room temperature, pour into cold water, filter. Wash the precipitate with water and recrystallize it from EtOH:benzene 50:50 (Caution! Benzene is a carcinogen!) to give 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole as a colorless powder (mp >300°) (J. Chromatogr. 1993, 619, 1).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Daisopak SP-120-ODS (Daiso, Osaka)
Mobile phase: Gradient. A was MeCN. B was MeCN:200 mM pH 7.0 Tris-HCl buffer 20:80. A:B 25:75 for 12 min, to 45:55 over 10 min, to 100:0 over 5 min, maintain at 100:0 for 10 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 475

CHROMATOGRAM

Retention time: 7.3
Internal standard: cyclohexanoylcarnitine (Synthesis of cyclohexanoylcarnitine is as follows. Dissolve 300 mg carnitine in 500 μL trifluoroacetic acid, add 1 mL cyclohexanecarbonyl chloride, protect from moisture with a calcium chloride tube, mix, heat at 40-45° overnight, cool to room temperature, add 5 mL acetone, cool on ice for a couple of h, centrifuge to remove undissolved material, add diethyl ether to incipient cloudiness, when crystallization starts add 10 mL diethyl ether, cool on ice. Dissolve the crystallization products in 1 mL MeOH, add 4-5 mL acetone, add diethyl ether to incipient cloudiness, when crystallization starts add 5 mL diethyl ether (cf. Biochim. Biophys. Acta 1968, 152, 559) to obtain cyclohexanoylcarnitine hydrochloride (mp 159-160°).) (20)
Limit of detection: 240 nM

OTHER SUBSTANCES

Extracted: acetylcarnitine, hexanoylcarnitine, octanoylcarnitine, propionylcarnitine

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Kuroda,N.; Ohyama,Y.; Nakashima,K.; Akiyama,S. HPLC determination of carnitine and acylcarnitines in human plasma by means of fluorescence labeling using 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole, *Chem.Pharm.Bull.*, **1996**, *44*, 1525–1529.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg SAX-Isolute SPE cartridge (Stepbio, Bologna) with 500 μL MeOH and 1 mL water. 100 μL Plasma + 30 μL 17.6 μg/mL methanesulfonyl-L-carnitine in water containing 640 ng/mL isobutyryl-L-carnitine + 370 μL water, add to the SPE cartridge, elute with 500 μL 10 mM pH 3.5 phosphate buffer. With continuous vortexing add 20 μL 1 M HCl, 100 μL 16 mg/mL 1-aminoanthracene in acetone, and 100 μL 160 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in 10 mM pH 3.5 NaH_2PO_4 buffer (in 20 μL aliquots) to the eluate, let stand at 25° for 20 min, wash with 5 mL diethyl ether. Remove a 300 μL aliquot of the aqueous phase and add it to 700 μL 10 mM pH 9.1 Na_2HPO_4 buffer, wash with 5 mL chloroform. Remove a 500 μL aliquot of the aqueous phase and add it to 500 μL 10 mM pH 3.5 NaH_2PO_4 buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Kromasil C18
Mobile phase: MeCN:100 mM pH 3.5 ammonium acetate 30:70
Flow rate: 1.3
Injection volume: 20
Detector: F ex 248 em 418

CHROMATOGRAM
Retention time: 8
Internal standard: methanesulfonyl-L-carnitine (14.5), isobutryl-L-carnitine (21)
Limit of quantitation: 5 μM

OTHER SUBSTANCES
Extracted: acetylcarnitine, propionylcarnitine

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Longo,A.; Bruno,G.; Curti,S.; Mancinelli,A.; Miotto,G. Determination of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in human plasma by high-performance liquid chromatography after pre-column derivatization with 1-aminoanthracene, *J.Chromatogr.B*, **1996**, *686*, 129–139.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Potter-Elvehjem) 200-300 mg tissue with 8 volumes cold 6% perchloric acid or mix plasma with two volumes of 6% perchloric acid, centrifuge at 5000 g for 10 min, wash pellet with 6% (tissue) or 3% (plasma) perchloric acid, centrifuge again, combine the supernatants, neutralize with KOH, allow to stand on ice for 30 min, centrifuge, filter (0.45 μm, Biofield chromatodisc 13 A). To a total volume of 1 mL add filtrate, 0.5 μmole EDTA, 10 μmole pH 7.5 phosphate buffer, 40 nmole acetylcoenzyme A, and 1 U carnitine acetyltransferase, incubate at 25° for 30 min, adjust pH to 2 with phosphoric acid, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Unisil QC8 (Gasukuro Kogyo)

Mobile phase: MeOH:190 mM KH_2PO_4 13:87
Flow rate: 0.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8 (of coenzyme A, the product of the reaction)

KEY WORDS
plasma; Guinea pig; liver; kidney; heart; muscle; enzymic reaction

REFERENCE
Arakawa,N.; Ha,T.Y.; Otsuka,M. An improved high-performance liquid chromatographic assay for the determination of free and esterified carnitine in animal tissues, *J.Nutr.Sci.Vitaminol.(Tokyo)*, **1989**, *35*, 475–479.

SAMPLE
Matrix: blood, urine
Sample preparation: Pack a disposable polypropylene chromatography column with 0.5 mL 230-400 mesh Silica gel 60 (Curtin Matheson). 25 μL Urine (or 10 μL urine + 20 μL water or 100 μL plasma) + 25 μL 100 μM IS + 25 μL 1 M KH_2PO_4 + 1 mL MeCN:MeOH 75:25, vortex for 2 s, centrifuge at 13600 g for 5 min, add the supernatant to the silica gel column, wash with 2 mL MeOH, wash with 1 mL 1% acetic acid in MeOH, elute with 4 mL 1% acetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 250 μL MeCN:MeOH 75:25, vortex for 2 s, centrifuge at 13600 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 10 μL diisopropylethylamine solution and 20 μL 100 mM 4'-bromophenacyl trifluoromethanesulfonate in MeCN, vortex for 2 s, let stand for 10 min, inject a 6 μL aliquot. (The diisopropylethylamine solution was 25 μL diisopropylethylamine in 10 mL MeOH. Prepare 4'-bromophenacyl trifluoromethanesulfonate as follows. Add 8.8 g p-bromobenzoyl chloride in 40 mL dry ether over 20-30 min to 100 mmoles diazomethane stirred in an ice bath, stir in an ice bath for 8-9 h, let stand at room temperature for 3 h, evaporate the solvent under reduced pressure, recrystallize 4'-bromo-2-diazoacetophenone from ether/hexane (mp 123.5-124° d) (J.Am.Chem.Soc. 1951, 73, 5301). Condense 50 mL anhydrous sulfur dioxide in a flask fitted with a calcium sulfate drying tube, cool in a dry ice/acetone bath, add 2.25 g 4'-bromo-2-diazoacetophenone, stir for 5 min, add 900 μL anhydrous trifluoromethanesulfonic acid from a freshly opened bottle in one portion, stir for 15 min, remove the cooling bath, after 30 min use an ice/water bath to evaporate the solvent. Dissolve the residue in 100 mL boiling dichloromethane, treat twice with 5 g portions of decolorizing carbon, filter, evaporate the filtrate, recrystallize the residue from pentane:dichloromethane 80:20 to give 4'-bromophenacyl trifluoromethanesulfonate as colorless plates (mp 137-8°) (J.Chromatogr. 1984, 299, 365).)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil (MOS-1) C8
Mobile phase: Gradient. A was MeCN:water 80:20. B was MeCN:water 20:80. C was MeCN:water:phosphoric acid:triethylamine 20:80:0.4:0.5. D was MeCN:water:phosphoric acid:triethylamine 90:10:0.2:0.25. A:B:C:D 100:0:0:0 for 0.2 min, 0:100:0:0 for 0.8 min, then 0:0:100:0 to 0:0:0:100 over 10 min, then 100:0:0:0 for 3 min before next run.
Flow rate: 1.75
Injection volume: 6
Detector: UV 260

CHROMATOGRAM
Retention time: 4.25
Internal standard: 4-(N,N-dimethyl-N-ethylammonio)-3-hydroxybutanoate (Prepare by N-demethylating carnitine and alkylating the resulting 4-(N,N-dimethylamino)-3-hydroxy-

butanoic acid with iodoethane. Perform the reaction under nitrogen and protect from light. Heat 83 g thiophenol and 20 g NaOH in 100 mL EtOH until they dissolve, add 700 mL toluene, distil slowly at atmospheric pressure, add 600 mL toluene in 100 mL portions to keep the volume in the flask at 500-600 mL, continue distillation for 1 h after the distillation head temperature reaches 115°. Sodium thiophenoxide crystallizes out in the flask as the distillation progresses (Anal.Chem. 1968, 40, 125). Filter the product under nitrogen and wash it with boiling toluene, store in the dark under vacuum. Dissolve 2 g l-carnitine chloride in 100 mL DMF with stirring at 80°, cool to room temperature, add 6.6 g sodium thiophenoxide, stir for 20 min, heat at 100° with stirring for 8 h, cool to room temperature, pour into 150 g ice and 4.5 mL concentrated HCl, wash 4 times with 100 mL portions of diethyl ether, evaporate the aqueous phase under reduced pressure at 40°, add more water to the flask until all the DMF is removed. When the volume has been reduced to 5 mL adjust pH to 10 with NaOH, add to a 300 × 20 column of 200-400 mesh Dowex 2X8 (OH^-) made up in 50 mM NaOH, wash with 200 mL 50 mM NaOH at 1.5 mL/min, wash with water until the pH of the effluent is neutral. Place the resin in a beaker containing ice water, slowly add concentrated HCl with stirring until the supernatant reaches pH 1, pour the resin into the column, wash the column with 200 mL 100 mM HCl. Collect all the effluents and concentrate them almost to dryness under reduced pressure, add to a 500 × 20 column of 200-400 mesh Dowex 50X8 (H^+ form), elute with 300 mL 500 mM HCl and 300 mL 2.5 M HCl, collect 10 mL fractions, identify fractions containing compound with iodoplatinate spray reagent. Combine fractions containing compound and lyophilize them to give 4-(N,N-dimethylamino)-3-hydroxybutanoic acid as a colorless glass. Dissolve 50 mg 4-(N,N-dimethylamino)-3-hydroxybutanoic acid and 150 mg barium hydroxide octahydrate in 1 mL water, add iodoethane in 5 mL MeOH, stir for 15 h, evaporate to dryness, reconstitute with 2 mL water, add 500 μL 1 M sulfuric acid, centrifuge, wash the solid with 100 mM sulfuric acid. Combine the aqueous layers and adjust the pH to 7 with KOH, add to a 190 × 12 column of 200-400 mesh Dowex 1X8 (OH^-), elute with water and collect fractions. Adjust the pH of the fractions containing the product to 4 with HCl, evaporate, add the residue to a 190 × 12 column of 200-400 mesh Dowex 50X8 (H^+) form, elute column with 60 mL 1 M HCl and 200 mL 2 M HCl, collect fractions (J.Labelled Cmpds.Radiopharm. 1982, 9, 535, J.Chromatogr. 1984, 336, 271, Clin.Chim.Acta 1992, 212, 55).) (4.5)

Limit of quantitation: 10 nmole/mL

OTHER SUBSTANCES

Extracted: carnitine esters, betaine, butyrobetaine, trimethyllysine

KEY WORDS

plasma; SPE; derivatization; plasma details (see Anal. Biochem. 1993; 212; 510)

REFERENCE

Minkler,P.E.; Hoppel,C.L. Quantification of carnitine and specific acylcarnitines by high-performance liquid chromatography: Application to normal human urine and urine from patients with methylmalonic aciduria, isovaleric acidemia or medium-chain acyl-CoA dehydrogenase deficiency, *J.Chromatogr.*, **1993**, *613*, 203–221.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 287.8

CHROMATOGRAM

Retention time: 16.058

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk
Sample preparation: React 50 μmoles carnitines, 2 mmoles 4-(2-aminoethylamino)-7-nitro-2,1,3-benzoxadiazole, and 35 mmoles 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in 100 μL pyridine:DMF 20:80, let stand at room temperature for 2 h, add 900 μL 10 mM HCl in MeOH:water 80:20, add to a 150 μL 0.06 mequiv Toyopak IC-SP S (Tosoh) SPE cartridge, wash with 5 mL 10 mM HCl:MeOH 50:50, elute with 3 mL 1 M pH 7.0 triethylamine acetate in MeOH. Evaporate the eluate to dryness, reconstitute with 1 mL 100 mM trifluoroacetic acid:DMF 20:80, inject an aliquot. (Preparation of 4-(2-aminoethylamino)-7-nitro-2,1,3-benzoxadiazole is as follows. Add 10 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 10 mL MeCN dropwise to a stirred solution of 550 μmoles ethylenediamine in MeCN over 30 min, stir at room temperature for 2 h, remove MeCN by evaporation under reduced pressure, acidify with 5% HCl, purify by reverse phase HPLC using a 10 mM HCl/MeCN gradient (no further details).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSKgel ODS 80Ts (Tosoh)
Mobile phase: Gradient. A was 10 mM trifluoroacetic acid in water. B was 10 mM trifluoroacetic acid in MeCN:water 90:10. A:B from 100:0 to 85:15 over 1 min, to 75:25 over 9 min, to 65:35 over 10 min, to 0:100 over 10 min, maintain at 0:100 for 2 min, return to initial conditions over 1 min.
Injection volume: 100
Detector: F ex 485 em 540

CHROMATOGRAM

Retention time: 8
Limit of detection: 100 fmole

OTHER SUBSTANCES

Simultaneous: acetylcarnitine, butyrylcarnitine, decanoylcarnitine, heptanoylcarnitine, hexanoylcarnitine, isobutyrylcarnitine, isovalerylcarnitine, lauroylcarnitine, myristoylcarnitine, nonanoylcarnitine, octanoylcarnitine, palmitoylcarnitine, propionylcarnitine, stearoylcarnitine, valerylcarnitine, valproylcarnitine

KEY WORDS
derivatization; SPE

REFERENCE
Matsumoto,K.; Ichitani,Y.; Ogasawara,N.; Yuki,H.; Imai,K. Precolumn fluorescence derivatization of carnitine and acylcarnitines with 4-(2-aminoethylamino)-7-nitro-2,1,3-benzoxadiazole prior to high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *678*, 241–247.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount containing 25 mg carnitine chloride, add 40 mL water, shake for 10 min, make up to 50 mL, centrifuge at 3000 rpm for 5 min, dilute supernatant five times. 10 μL Diluted supernatant + 10 μL 20% aqueous sodium dodecyl sulfate + 10 μL 100 μg/mL N,N-dimethylglycine in water + 370 μL isopropanol + 100 μL 10 mg/mL 9-anthryldiazomethane in ethyl acetate, heat at 50° for 30 min, cool, inject a 55 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 10 μm LiChrosorb Si 100
Mobile phase: MeOH:5% aqueous sodium dodecyl sulfate:phosphoric acid 99:1:0.1
Flow rate: 1
Injection volume: 55
Detector: F ex 365 em 412 or UV 250

CHROMATOGRAM
Retention time: 10
Internal standard: N,N-dimethylglycine (5)
Limit of detection: 1 pg

OTHER SUBSTANCES
Noninterfering: antacids, ethyl aminobenzoate, thiamine, riboflavin, pyridoxine, ascorbic acid, niacinamide, pantothenic acid, dicycloverine, papaverine, methylbenactyzium

KEY WORDS
derivatization; tablets; normal phase

REFERENCE
Yoshida,T.; Aetake,A.; Yamaguchi,H.; Nimura,N.; Kinoshita,T. Determination of carnitine by high-performance liquid chromatography using 9-anthryldiazomethane, *J.Chromatogr.*, **1988**, *445*, 175–182.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out an appropriate amount, add 40 mL water, sonicate for 20 min, cool, make up to 50 mL, centrifuge at 1300 g for 10 min. remove a 2 mL aliquot of the supernatant and add it to a 150 × 10 column containing 2 g 100-200 mesh Amberlite CG-120 cation-exchange resin (Na^+ form), wash with 25 mL water, elute with 20 mL 2% ammonia solution, adjust the volume of the eluate to 25 mL with water. Remove a 500 μL aliquot and add it to 500 μL 50 μg/mL triamterene in MeCN:DMSO 99:1, evaporate to dryness under reduced pressure at 50°, reconstitute with 1 mL 200 μg/mL pyrene-1-carbonyl cyanide (Wako Chemicals, Richmond VA) in DMSO, heat at 80° for 30 min, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSKgel SP-2SW (Tosoh)
Mobile phase: MeCN:buffer 25:75 (Buffer was 10 mM $(NH_4)_2HPO_4$ adjusted to pH 7.5 with phosphoric acid.)
Column temperature: 40

Flow rate: 1
Injection volume: 1
Detector: F ex 355 em 420

CHROMATOGRAM
Retention time: 5
Internal standard: triamterene (8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: caffeine, cinnamon bark extract, coptis rhizome extract, EtOH, gentian extract, ginseng extract, glucuronolactone, glycyrrhizia extract, inositol, niacinamide, pantothenol, pyridoxine, riboflavin, sucrose, thiamine

KEY WORDS
derivatization; tablets; SPE

REFERENCE
Kamata,K.; Takahashi,M.; Terasima,K.; Nishijima,M. Liquid chromatographic determination of carnitine by precolumn derivatization with pyrene-1-carbonyl cyanide, *J.Chromatogr.A*, **1994**, *667*, 113–118.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out syrup, injections, or finely ground tablets containing 50 mg carnitine, add 40 mL water, sonicate for 20 min, cool, make up to 50 mL with water, centrifuge at 2000 rpm for 10 min. Remove a 1 mL aliquot and add it to 1 mL 100 μg/mL quinuclidine in MeCN, evaporate to dryness under reduced pressure at 50°, add 1 mL 2 mg/mL 9-anthroylnitrile (Wako) in DMSO, heat at 80° for 1.5 h, cool to room temperature, make up to 5 mL with DMSO, add a 100 μL aliquot to a 1 mL Bond Elut silica gel SPE cartridge, wash with 10 mL MeCN:MeOH 90:10, elute with 20 mL water, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Ultron ES-OVM ovomucoid-conjugated (Shinwa, Kyoto)
Mobile phase: MeCN:buffer 17:83 (Buffer was 20 mM KH_2PO_4 adjusted to pH 4.5 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7 (D), 10 (L)
Limit of detection: 50 μg/mL

KEY WORDS
derivatization; chiral; SPE; tablets; syrup; injections

REFERENCE
Takahashi,M.; Terashima,K.; Nishijima,M.; Kamata,K. Separation of carnitine enantiomers as the 9-anthroylnitrile derivatives and high-performance liquid chromatographic analysis on an ovomucoid-conjugated column, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1579–1584.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil NH2
Mobile phase: MeCN:20 mM pH 3 phosphate buffer 75:25
Flow rate: 1
Detector: UV 205

CHROMATOGRAM
Retention time: 8.65
Limit of quantitation: 185 μM

OTHER SUBSTANCES
Simultaneous: propionyl L-carnitine, crotonylbetaine, propionic acid

REFERENCE
Marzo,A.; Monti,N.; Ripamonti,M.; Arrigoni Martelli,E. Application of high-performance liquid chromatography to the analysis of propionyl-L-carnitine by a stereospecific enzyme assay, *J.Chromatogr.*, **1988**, *459*, 313–317.

SAMPLE
Matrix: solutions
Sample preparation: 50 μL 200 μg/mL carnitine in 50 mM aqueous tetrabutylammonium phosphate + 200 μL 4.5 mM (+)-1-(9-fluorenyl)ethyl chloroformate in acetone, heat at 80° in a sealed vial for 25 min, cool, dilute with 4 mL MeCN:buffer 25:75, inject a 5 μL aliquot. (Buffer was 5 mM tetrabutylammonium hydroxide and 50 mM KH_2PO_4 adjusted to pH 7.0 with 1 M KOH.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN:buffer 25:75. B was MeCN:5 mM KH_2PO_4 75:25. A:B 100:0 for 20 min, to 0:100 over 2 min, maintain at 0:100 for 13 min, to 100:0 over 1 min, maintain at 100:0 for 4 min. (Buffer was 5 mM tetrabutylammonium hydroxide and 50 mM KH_2PO_4 adjusted to pH 7.0 with 1 M KOH.)
Column temperature: 30
Flow rate: 0.75 for 20 min, then 1
Injection volume: 5
Detector: UV 260 or F ex 260 em 315

CHROMATOGRAM
Retention time: 12.5 (D), 14.5 (L)

KEY WORDS
derivatization

REFERENCE
De Witt,P.; Deias,R.; Muck,S.; Galletti,B.; Meloni,D.; Celletti,P.; Marzo,A. High-performance liquid chromatography and capillary electrophoresis of L- and D-carnitine by precolumn diastereomeric derivatization, *J.Chromatogr.B*, **1994**, *657*, 67–73.

SAMPLE
Matrix: solutions
Sample preparation: 500 μL 5 mg/mL carnitine in water + 100 mL acetone, remove a 1 mL aliquot and add it to 250 μL 1 mg/mL 9-anrthryldiazomethane in acetone, let stand at 50° for 20 min, evaporate the solvent under a stream of nitrogen, add 2 mL 0.1% perchloric acid to the residue, wash this solution twice with 6 mL diethyl ether. Remove 1 mL of the aqueous phase and add it to 1 mL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD-R (tris(3,5-dimethylphenylcarbamate)

Mobile phase: MeCN:500 mM sodium perchlorate solution 40:60
Flow rate: 0.8
Injection volume: 20
Detector: UV 254 or F ex 365 em 412

CHROMATOGRAM
Retention time: 16 (D), 18 (L)

KEY WORDS
derivatization; also for carnitine esters

REFERENCE
Hirota,T.; Minato,K.; Ishii,K.; Nishimura,N.; Sato,T. High-performance liquid chromatographic determination of the enantiomers of carnitine and acetylcarnitine on a chiral stationary phase, *J.Chromatogr.A*, **1994**, *673*, 37–43.

SAMPLE
Matrix: solutions
Sample preparation: 30 μL Carnitine in water + 30 μL 50 mM pH 10.4 carbonate buffer + 80 μL 15 mM (+)-[1-(9-fluorenyl)ethyl]chloroformate in acetone, heat at 45° for 1 h, add 90 μL 50 mM pH 4.2 acetate buffer, inject an aliquot.

HPLC VARIABLES
Column: 240 × 4.6 5 μm RP18
Mobile phase: MeCN:buffer 28.5:71.5 (Buffer was 6.8 mL triethylamine in 1 L water, adjust pH to 2.6 with 85% phosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: F ex 260 em 310

CHROMATOGRAM
Retention time: 15 (D), 17 (L)
Limit of detection: 0.5% of other enantiomer

KEY WORDS
chiral; derivatization

REFERENCE
Vogt,C.; Georgi,A.; Werner,G. Enantiomeric separation of D/L-carnitine using HPLC and CZE after derivatization, *Chromatographia*, **1995**, *40*, 287–295.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 250 μL 200 μM IS + 125 μL 1 M KOH, let stand for 10 min, add to the column, elute with 2.5 mL 500 mM ammonium hydroxide. Evaporate the eluate to dryness, reconstitute the residue in 100 μL 3 mM N,N-diisopropylethylamine in MeCN, vortex for 2 min, add 100 μL 7.5 mM 4'-bromophenacyl triflate in MeCN, vortex for 2 min, inject a 20 μL aliquot. (The column was 35 × 5 Dowex 50-X8 (NH_4^+ form) above 35 × 5 Dowex 1-X8 (OH^- form) in a Pasteur pipette. Convert Dowex 50-X8 (200-400 mesh, H^+ form) to the NH_4^+ form and 35 × 5 Dowex 1-X8 (200-400 mesh, Cl^- form) to the OH^- form according to instructions from Bio-Rad Labs. In particular conversion of Dowex 1-X8 must continue until tests for Cl^- in the column effluent are negative.)

HPLC VARIABLES
Guard column: 50 × 4 Co:Pell ODS
Column: 100 × 5 10 μm Radial-Pak C18

Mobile phase: MeCN:buffer 58:22 (The buffer was 3.56 g sodium dodecyl sulfate, 2.21 g KH_2PO_4, and 4.75 mL 3-(dimethylamino)-1,2-propanediol in 2.2 L water, adjust pH to 6.5 with concentrated phosphoric acid.)
Flow rate: 3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: 4-(N,N-dimethyl-N-(n-propyl)ammonio)-3-hydroxybutanoate (11.5)

OTHER SUBSTANCES
Extracted: butyrobetaine

KEY WORDS
SPE; derivatization

REFERENCE
Minkler,P.E.; Ingalls,S.T.; Hoppel,C.L. Determination of total carnitine in human urine by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 385–393.

SAMPLE
Matrix: urine
Sample preparation: Condition a 500 mg silica SPE cartridge (Baker) with 5 mL MeOH. 500 μL Urine + 100 nmoles IS in water, evaporate to dryness under reduced pressure, reconstitute with 500 μL MeOH, centrifuge at 10000 g for 3 min, add the supernatant to the SPE cartridge, wash with 2 mL MeOH, elute with 3 mL MeOH:water:acetic acid 45:50:5. Collect the final 2.5 mL of eluate and evaporate it to dryness under reduced pressure, reconstitute with 500 μL MeOH, centrifuge. Evaporate the supernatant to dryness under a stream of nitrogen in a clean tube at 40°, add 300 μL 6.8 mM N,N-diisopropylethylamine in MeCN, vortex for 10 s, sonicate for 15 min, add 200 μL 25 mM 4'-bromophenacyl bromide in MeCN, vortex for 10 s, heat at 37° for 30 min, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Hypersil BDS C8
Column: 200 × 4.6 5 μm Hypersil BDS C8
Mobile phase: Gradient. A was MeCN:water 70:30. B was MeCN:100 mM pH 5.0 triethylamine phosphate buffer 95:5. A:B 97:3 for 9 min, to 90:10 over 3 min, to 50:50 over 8 min, to 10:90 over 6 min, maintain at 10:90 for 3 min, return to initial conditions over 10 min, re-equilibrate for 5 min
Flow rate: 1.2 for 12 min, then 1.4
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 14
Internal standard: undecanoyl-L-carnitine (24)

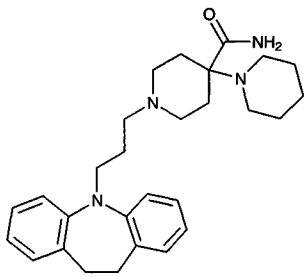
OTHER SUBSTANCES
Extracted: acetylcarnitine, butyrylcarnitine, decanoylcarnitine, hexanoylcarnitine, isovalerylcarnitine, nonanoylcarnitine, octanoylcarnitine, palmitoylcarnitine, propionylcarnitine

KEY WORDS
derivatization; SPE

REFERENCE

Poorthuis,B.J.H.M.; Jille-Vlckov,T.; Onkenhout,W. Determination of acylcarnitines in urine of patients with inborn errors of metabolism using high-performance liquid chromatography after derivatization with 4'-bromophenacylbromide, *Clin.Chim.Acta*, **1993**, *216*, 53–61.

Carpipramine



Molecular formula: $C_{28}H_{38}N_4O$
Molecular weight: 446.64
CAS Registry No.: 5942-95-0, 7075-03-8 (dihydrochloride monohydrate)
Merck Index: 1911
Lednicer No.: 2 416

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 251

CHROMATOGRAM

Retention time: 12.58
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine;

aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.18

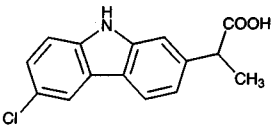
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Carprofen



Molecular formula: $C_{15}H_{12}ClNO_2$
Molecular weight: 273.72
CAS Registry No.: 53716-49-7
Merck Index: 1912
Lednicer No.: 2 169

SAMPLE

Matrix: bile, blood, urine

Sample preparation: Bile. Centrifuge sample, dilute 10-fold with 1% phosphoric acid, inject a 20 μL aliquot. Plasma. Vortex plasma, centrifuge, apply a 100-500 μL aliquot to a Varian C18 SPE cartridge. Wash with 500 gmL 1% phosphoric acid, wash with 500 μL MeOH: 1% phosphoric acid 20:80, elute with MeOH (?). Urine. Vortex urine, centrifuge, add a 500 μL aliquot to a Varian C18 SPE cartridge. Wash with 500 gmL 1% phosphoric acid, wash with 500 μL MeOH: 1% phosphoric acid 40:60, elute with MeOH (?).

HPLC VARIABLES

Column: 250 × 4.6 5 μm RP18 Kromasil (Interchim, France)

Mobile phase: Gradient. A. MeCN. B. 20 mM pH 5 NA_2HPO_4, 2 H_2O containing 10 mM citric acid. A:B 27:73 for 6 min, to 40:60 over 10 min.

Detector: F ex 310 em 375

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Extracted: carprofen glucuronides

KEY WORDS

plasma; dog; pharmacokinetics; SPE

REFERENCE

Priymenko,N.; Garnier,F.; Ferre,J.-P.; Delatour,P.; Toutain,P.-L. Enantioselectivity of the enterohepatic recycling of carprofen in the dog, *Drug Metab.Dispos.*, **1998**, *26*, 170–176.

SAMPLE

Matrix: blood

Sample preparation: Vortex plasma, centrifuge, apply a 100-500 μL aliquot to a Varian C18 SPE cartridge. Wash with 1 mL MeOH:water 20:80, elute with 1 mL MeOH. Evaporate the eluate to dryness, add 50 μL toluene, evaporate to dryness under nitrogen, reconstitute in 100 μL 50 mM triethylamine in dry MeCN, let stand for 1 min, add 50 μL 60 mM ethyl chloroformate in dry MeCN, mix, let stand for 1 min, add 50 μL 1 M L-leucinamide HCl in MeOH containing 1 M triethylamine, mix, let stand for 3 min, add 200 μL 250 mM HCl, extract with 4 mL ethyl acetate (J. Chromatogr. 1988, 433, 331), reconstitute with MeOH, pass through the same SPE cartridge, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm RP18 Kromasil (Interchim, France)

Mobile phase: Gradient. A. MeCN. B. 20 mM pH 5 NA_2HPO_4, 2 H_2O containing 10 mM citric acid. A:B from 40:60 to 60:40 over 12 min.

Detector: F ex 310 em 375 LQ 150 ng/mL

CHROMATOGRAM

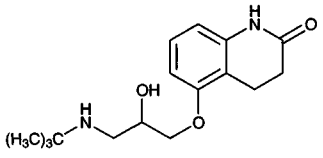
Retention time: 10 (R), 10.7 (S)

KEY WORDS
plasma; dog; chiral; pharmacokinetics; derivatization; SPE

REFERENCE

Priymenko,N.; Garnier,F.; Ferre,J.-P.; Delatour,P.; Toutain,P.-L. Enantioselectivity of the enterohepatic recycling of carprofen in the dog, *Drug Metab.Dispos.*, **1998**, *26*, 170–176.

Carteolol



Molecular formula: $C_{16}H_{24}N_2O_3$
Molecular weight: 292.38
CAS Registry No.: 51781-06-7, 51781-21-6 (HCl)
Merck Index: 1917
Lednicer No.: 3 183

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 251

CHROMATOGRAM

Retention time: 3.60

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Deproteinize plasma with perchloric acid, extract with dichloromethane, make extract alkaline with NaOH, extract with chloroform. Urine. Acidify urine with HCl, extract with chloroform, make extract alkaline with NaOH, extract with chloroform.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM $(NH_4)H_2PO_4$ and 20 mM $(NH_4)_2HPO_4$.)

Detector: UV 254

CHROMATOGRAM

Internal standard: 1-methylcarteolol

Limit of quantitation: 200 ng/mL (urine), 5 ng/mL (plasma)

KEY WORDS

plasma

REFERENCE

Odomi,M.; Akiyama,H.; Matsuura,K.; Shimizu,T. [The determination of carteolol in human plasma and urine by high performance liquid chromatography], *Yakugaku.Zasshi.*, **1985**, *105*, 459–463.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM
Retention time: 5.935

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL 20% sodium bicarbonate containing 500 μg/mL IS and 20 mg/mL sodium bisulfite, mix. Extract the mixture with 5 mL ethyl acetate. Remove the organic layer and dry it under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase. Inject a 20-30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80Ts (Tosoh Co., Japan)
Mobile phase: MeCN:water:acetic acid 10.5:89.5:1
Flow rate: 0.8
Injection volume: 20-30
Detector: UV 254

CHROMATOGRAM
Retention time: 17.7
Internal standard: p-aminobenzoic acid (11.7)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Umehara,K.; Kudo,S.; Odomi,M. Involvement of CYP2D1 in the metabolism of carteolol by male rat liver microsomes, *Xenobiotica*, **1997**, *27*, 1121–1129.

SAMPLE
Matrix: perfusate
Sample preparation: 50 μL Perfusate + 50 μL pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 μL 20 μg/mL pindolol in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 25:75

Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Internal standard: pindolol

KEY WORDS
rabbit

REFERENCE
Sasaki,H.; Igarashi,Y.; Nagano,T.; Nishida,K.; Nakamura,J. Different effects of absorption promoters on corneal and conjunctival penetration of ophthalmic β-blockers, *Pharm.Res.*, **1995**, *12*, 1146–1150.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 internal surface reversed-phase silica (Pinkerton) (Regis Chemical)
Mobile phase: Isopropanol:100 mM pH 6.8 KH_2PO_4 10:90
Flow rate: 1
Injection volume: 10
Detector: UV 232-274 (wavelength of maximum absorption used)

CHROMATOGRAM
Retention time: 32.6

OTHER SUBSTANCES
Simultaneous: atenolol, metoprolol, acebutolol, oxprenolol, pindolol, alprenolol

REFERENCE
Ohshima,T.; Takagi,K.; Miyamoto,K.-I. High performance liquid chromatographic retention time of β-blockers as an index of pharmacological activity, *J.Liq.Chromatogr.*, **1993**, *16*, 3933–3939.

SAMPLE
Matrix: solutions
Sample preparation: 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL 20 μg/mL pindolol in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 25:75
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Internal standard: pindolol

KEY WORDS
buffer; earle's balanced salt solution

REFERENCE
Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 60:40:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: k' 0.48, 0.95 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.71

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g

powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/ 12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation

under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 35:65:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 23.4, 28.3 (enantiomers)
Limit of detection: 0.00635-0.008 fmole

OTHER SUBSTANCES

Also analyzed: atenolol, timolol

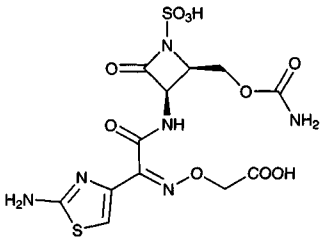
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Carumonam



Molecular formula: $C_{12}H_{14}N_6O_{10}S_2$
Molecular weight: 466.41
CAS Registry No.: 87638-04-8, 86832-68-0 (sodium salt)
Merck Index: 1922
Lednicer No.: 4 193

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 250 μL 100 mM pH 3.0 citrate buffer, vortex for 10 s, add 1 mL MeCN, vortex twice for 15 s, centrifuge at 1000 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex for 15 s, centrifuge at 1000 g for 10 min. Remove 200 μL of the aqueous phase and add it to 50 μL mobile phase, remove residual dichloromethane by evaporating under reduced pressure in a rotary evaporator (5 min at 200 Torr), inject a 40 μL aliquot into column A and column B in series, after 3.5 min remove column A from the circuit and backflush it with mobile phase to remove late-eluting peaks. Urine. 50 μL Urine + 200 μL 100 mM pH 5 phosphate buffer + 1 mL mobile phase, vortex, inject a 20 μL aliquot into column A and column B in series, after 3.5 min remove column A from the circuit and backflush it with mobile phase to remove late-eluting peaks.

HPLC VARIABLES

Column: A 30 × 4 5 μm Hypersil MOS; B 125 × 4 5 μm Hypersil ODS
Mobile phase: MeCN containing 4.5 g/L trihexylamine:water:50% sulfuric acid 30:70:0.1, adjusted to an apparent pH of 4.5 with 1 M NaOH
Flow rate: 1
Injection volume: 20-40
Detector: UV 293

CHROMATOGRAM

Retention time: 15
Limit of quantitation: 25 μg/mL (urine), 500 ng/mL (plasma)

KEY WORDS

plasma; column-switching

REFERENCE

Egger,H.-J.; Fischer,G. Determination of the monocyclic β-lactam antibiotic carumonam in plasma and urine by ion-pair and ion-suppression reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 357–372.

SAMPLE

Matrix: cell suspensions
Sample preparation: Filter (0.45 μm).

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere IP ion pair
Mobile phase: MeOH:100 mM sodium perchlorate adjusted to pH 2.5 with concentrated sulfuric acid 35:65
Flow rate: 1
Injection volume: 20
Detector: UV 295

CHROMATOGRAM

Retention time: 5.0

OTHER SUBSTANCES
Extracted: cefpirome (UV 254)
Interfering: ceftriaxone (UV 254), cefotaxime (UV 254)

REFERENCE
Bellido,F.; Pechère,J.-C.; Hancock,R.E.W. Novel method for measurement of outer membrane permeability to new β-lactams in intact *Enterobacter cloacae* cells, *Antimicrob.Agents Chemother.*, **1991**, *35*, 68–72.

SAMPLE
Matrix: dialysate, ultrafiltrate, urine
Sample preparation: No details given.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5C18
Mobile phase: MeCN:5 mM pH 3.0 tetrabutylammonium hydrogen sulfate 12:88
Flow rate: 0.8
Detector: UV 313

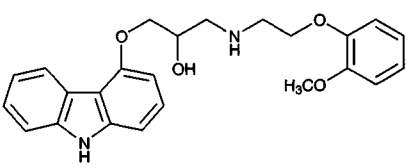
CHROMATOGRAM
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; rat; monkey; rabbit; dog

REFERENCE
Kita,Y.; Fugono,T.; Imada,A. Comparative pharmacokinetics of carumonam and aztreonam in mice, rats, rabbits, dogs, and cynomolgus monkeys, *Antimicrob.Agents Chemother.*, **1986**, *29*, 127–134.

Carvedilol



Molecular formula: $C_{24}H_{26}N_2O_4$
Molecular weight: 406.48
CAS Registry No.: 72956-09-3
Merck Index: 1924
Lednicer No.: 5 163

SAMPLE

Matrix: blood
Sample preparation: Condition a PrepSep RC18 SPE cartridge (Fisher) with 10 mL elution solvent and 2 mL MeCN:water 35:65. 1 mL Plasma + 1 mL 8 M guanidine hydrochloride, mix, add to SPE cartridge, wash with 2 mL MeCN:water 35:65, elute with two 200 μL portions of elution solvent. Add 50 μL 100 mM triethylamine in MeCN and 10 μL 20 mg/mL 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate in MeCN to the eluate, vortex briefly, allow to stand at room temperature for 30 min, add 150 μL 0.5% phosphoric acid, inject a 50-100 μL aliquot. (Elution solvent was MeCN:water:1 M triethylamine in water adjusted to pH 2.5 with phosphoric acid 80:17:3.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:water:1 M triethylamine in water adjusted to pH 2.5 with phosphoric acid 29:29:41.5:0.5
Flow rate: 1.6
Injection volume: 50-100
Detector: F ex 285 em 360

CHROMATOGRAM

Retention time: 9.5 (S-(-)), 11.5 (R-(+))
Internal standard: N,N-bis-carvedilol (4)
Limit of detection: 0.6 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; chiral; SPE; derivatization

REFERENCE

Eisenberg,E.J.; Patterson,W.R.; Kahn,G.C. High-performance liquid chromatographic method for the simultaneous determination of the enantiomers of carvedilol and its O-desmethyl metabolite in human plasma after chiral derivatization, *J.Chromatogr.*, **1989**, *493*, 105–115.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 ng IS in MeOH + 1 mL 100 mM pH 8 Britton-Robinson buffer + 5 mL ether, shake for 10 min at ca. 60 strokes/min, centrifuge at 1500 g for 10 min. Remove the organic layer and add it to 300 μL 50 mM sulfuric acid, shake, centrifuge. Remove the aqueous layer and add it to 1 mL 100 mM pH 8 Britton-Robinson buffer, add 5 mL ether, shake for 10 min, centrifuge. Remove the organic layer and dry it over 300 mg $MgSO_4.7H_2O$ (sic) by vortexing for a few s and standing for 10 min. Evaporate the ether solution to dryness under a stream of nitrogen. Take up the residue in 200 μL MeCN:triethylamine 99.6:0.4 and add 5 μL 25.2 mM 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, let stand at room temperature for 10 min, evaporate under

a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm ODS (Brownlee)
Column: 150 × 4.6 5 μm ODS 80TM (Tosoh)
Mobile phase: MeOH:EtOH:2 mM pH 7.0 $(NH_4)_2HPO_4$ 60:4:36
Column temperature: 50
Flow rate: 2.5
Injection volume: 50
Detector: F ex 285 em 355

CHROMATOGRAM
Retention time: 5.4 (R-(+)), 6.3 (S-(-))
Internal standard: 1-(o-methoxyphenyl)-4-[3-(naphthyloxy)-2-hydroxypropyl]piperazine (7.8)
Limit of detection: 1.55 ng/mL

KEY WORDS
plasma; human; monkey; derivatization; pharmacokinetics

REFERENCE
Fujimaki,M.; Murakoshi,Y.; Hakusui,H. Assay and disposition of carvedilol enantiomers in humans and monkeys: evidence of stereoselective presystemic metabolism, *J.Pharm.Sci.*, **1990**, *79*, 568–572.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL pH 9.8 buffer + 500 mg NaCl + 5 mL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 30 min, centrifuge at 4000 g for 10 min. Remove a 4 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL toluene, evaporate to dryness, add 100 μL 400 μg/mL S-naproxen chloride in anhydrous dichloromethane, heat at 50° for 1 h, evaporate to dryness, reconstitute with 120 μL mobile phase, inject a 100 μL aliquot. Urine. 1 mL Urine (or plasma) + 1 mL pH 5 sodium citrate buffer + 10 μL (5.500 Fishman units) β-glucuronidase, heat at 37° for 4 h, add 200 μL 100 mM NaOH, add 1 mL buffer, add 500 mg NaCl, add 5 mL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 30 min, centrifuge at 4000 g for 10 min. Remove a 4 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL toluene, evaporate to dryness, add 100 μL 400 μg/mL S-naproxen chloride in anhydrous dichloromethane, heat at 50° for 1 h, evaporate to dryness, reconstitute with 120 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by mixing 42 mL 100 mM sodium carbonate with 58 mL 100 mM sodium bicarbonate, pH 9.8. Synthesis of S-naproxen chloride is as follows. Protect all compounds from light. Dissolve 500 mg naproxen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 1 h, evaporate to dryness under reduced pressure, dry over KOH under vacuum overnight to obtain S-naproxen chloride (mp 96°) (Arch. Pharm. (Weinheim) 1988, 321, 847).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: n-Hexane:dichloromethane:EtOH 112:36:1.7
Flow rate: 1.5
Injection volume: 100
Detector: F ex 285 em 355

CHROMATOGRAM
Retention time: k' 13.3 (S-(-)), k' 15.7 (R-(+))
Limit of detection: 1 ng/mL

KEY WORDS
derivatization; plasma; chiral; normal phase; pharmacokinetics

REFERENCE
Spahn,H.; Henke,W.; Langguth,P.; Schloos,J.; Mutschler,E. Measurement of carvedilol enantiomers in human plasma and urine using S-naproxen chloride for chiral derivatization, *Arch.Pharm.(Weinheim)*, **1990**, *323*, 465–469.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
. . .**Detector:** UV 254

CHROMATOGRAM
Retention time: k' 6.11, k' 7.35 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, atenolol, carazolol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Inject directly.

HPLC VARIABLES
Column: 250 × 4.6 Brownlee RP-300 C8

Mobile phase: Gradient. A was 100 mM pH 5.0 ammonium acetate. B was MeCN:water 80:20. A:B from 90:10 to 55:45 over 70 min, to 0:100 over 5 min, maintain at 0:100 for 5 min.
Flow rate: 1
Detector: UV 285

CHROMATOGRAM
Retention time: 60

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
dog; rat; liver

REFERENCE
Schaefer,W.H. Formation of a carbamoyl glucuronide conjugate of carvedilol in vitro using dog and rat liver microsomes, *Drug Metab.Dispos.*, **1992**, *20*, 130–133.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Centrifuge mixture at 1000 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Capcell Pak C18 AG120 (Shiseido)
Mobile phase: MeOH:100 mM pH 9.0 phosphate buffer containing 4% triethylamine 55:45
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 330 em 380

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 28 nmole/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Fujimaki,M. Oxidation of R(+) and S(-)-carvedilol by rat liver microsomes. Evidence for stereoselective oxidation and characterization of cytochrome P450 isozymes involved, *Drug Metab.Dispos.*, **1994**, *22*, 700–708.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in 50 μL DMF, make up to 500 μL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OF
Mobile phase: n-Hexane:isopropanol 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 50

Detector: UV 254

CHROMATOGRAM
Retention time: 9.5 (R-(+)), 18.3 (S-(-))

KEY WORDS
chiral

REFERENCE
Fujimaki,M.; Shintani,S.; Hakusui,H. Stereoselective metabolism of carvedilol in the rat. Use of enantiomerically radiolabeled pseudoracemates, *Drug Metab.Dispos.*, **1991**, *19*, 749–753.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 40:60 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 3.33 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 13.96

OTHER SUBSTANCES
Also analyzed: bisoprolol, labetalol, metipranolol, oxprenolol, talinolol, toliprolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a

short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES

Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 100
Detector: UV 230, F ex 270 em 350

CHROMATOGRAM

Retention time: k' 7.1 (S-(-)), 8.0 (R-(+))

OTHER SUBSTANCES

Simultaneous: flecainide (no enantiomeric separation), propafenone

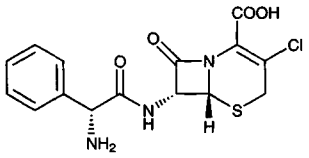
KEY WORDS

derivatization; chiral; F not much more sensitive than UV; α = 1.13

REFERENCE

Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

Cefaclor



Molecular formula: $C_{15}H_{14}ClN_3O_4S$
Molecular weight: 367.81
CAS Registry No.: 53994-73-3, 70356-03-5 (monohydrate)
Merck Index: 1962
Lednicer No.: 3 209

SAMPLE

Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 2

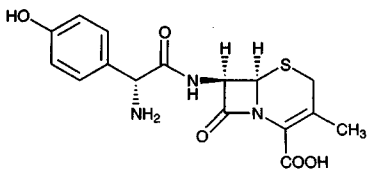
OTHER SUBSTANCES

Simultaneous: 7-ACA, 7-ADCA, cefaloridine, cefazolin, cefoperazone, cefotaxime, ceftazidime, cephalosporin C

REFERENCE

Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

Cefadroxil



Molecular formula: $C_{16}H_{17}N_3O_5S$
Molecular weight: 363.39
CAS Registry No.: 66592-87-8
Merck Index: 1963
Lednicer No.: 2 440

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL water + 200 μL 1 M pH 5 acetate buffer, vortex briefly. Filter (Centrifree micropartition unit) 1 mL mixture while centrifuging at 2000 g for 10 min. Inject a 25 μL aliquot of the ultrafiltrate. Urine. 1 mL urine + 1 mL 10 mM pH 3.5 acetate buffer + 1 mL 10% tetrabutylammonium hydroxide (adjusted to pH 2 with sulfuric acid) + 7.5 mL water saturated mixture of n-amyl alcohol:dichloromethane 2:1, mix on a flat-bed shaker at 100 strokes/min for 15 min, centrifuge at 2500 g for 5 min. Remove organic phase and add 7.5 mL water saturated mixture of n-amyl alcohol:dichloromethane 2:1. Briefly vortex a 250 μL aliquot of the upper aqueous phase with 4.75 mL water. Inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleogil C18
Mobile phase: MeCN:10 mM phosphoric acid adjusted to pH 3 with NaOH 6:94 (plasma) or MeCN:10 mM pH 3.0 phosphoric acid 5:95 (urine)
Flow rate: 1
Injection volume: 25
Detector: UV 260

CHROMATOGRAM

Retention time: 5.6 (plasma) or 7.0 (urine)
Limit of quantitation: 200 ng/mL (plasma), 10 μg/mL (urine)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Barbhaiya,R.H. A pharmacokinetic comparison of cefadroxil and cephalexin after administration of 250, 500 and 1000 mg solution doses, *Biopharm.Drug Dispos.*, **1996**, *17*, 319–330.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 150 μL Plasma + 150 μL MeCN, vortex, rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min. Transfer supernatant to another tube and add 7 volumes dichloromethane, equilibrate for 10 min; rotate at 20 rpm for 10 min; centrifuge at 1000 g for 10 min, inject an aliquot of the upper aqueous layer (J.Chromatogr. 1987, 413, 109). Urine. Dilute with water, inject an aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 1.6 Spherisorb S5-ODS2 C18
Mobile phase: MeOH:100 mM pH 3 acetate buffer 13:87
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Limit of detection: 300 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Gimeno,M.J.; Martínez,M.; Granero,L.; Torres-Molina,F.; Peris,J.-E. Influence of probenecid on the renal excretion mechanisms of cefadroxil, *Drug Metab.Dispos.*, **1996**, *24*, 270–272.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.188

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: React a sample with 300 mM HCl at 24° for 2 h or with 10 mM NaOH at ambient temperature for 1.5 h or with 1 M NaOH for 0.5 h or by heating the solid at 140° for 6 h or by heating a solution at 40° for 8 h.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeCN. B was 2 mM ammonium acetate. A:B 2:98 for 30 min, from 2:98 to 20:80 in 10 min, from 20:80 to 50:50 in 10 min
Flow rate: 1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 18.31

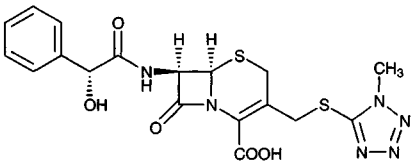
OTHER SUBSTANCES

Simultaneous: degradation products

REFERENCE

Rourick,R.A.; Volk,K.J.; Klohr,S.E.; Spears,T.; Kerns,E.H.; Lee,M.S. Predictive strategy for the rapid structure elucidation of drug degradants, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1743–1752.

Cefamandole



Molecular formula: $C_{18}H_{18}N_6O_5S_2$
Molecular weight: 462.51
CAS Registry No.: 34444-01-4, 42540-40-9 (nafate), 30034-03-8 (sodium)
Merck Index: 1964
Lednicer No.: 2 441

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 7.8 (mobile phase A), 10.3 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 8.2
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 15 μg/mL

OTHER SUBSTANCES
Extracted: cefazolin, cefotaxime, cefoxitin, cephapirin, chloramphenicol
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 19.2:0.8:80, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: cephalothin (8)
Limit of detection: 1000 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Cool blood in ice. 1 mL Blood + 100 μL pH 5.0 sodium acetate buffer + 100 μL 0.05% tri-o-tolyl phosphate in MeOH, centrifuge, remove plasma, add 10 μL glacial acetic acid. 200 μL Plasma + 200 μL 20 μg/mL cephalothin in 50 mM phosphoric acid, mix, keep at 4° before injection, inject a 100 μL aliquot onto column A with mobile phase A, elute column A with mobile phase A for 10 min, backflush the contents of column A onto column B with mobile phase B for 4 min, remove column A from the circuit, continue to elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 15 min. Urine. 1.5 mL Urine + 0.5 mL pH 3.0 2 M sodium acetate buffer. Dilute with 20 μg/mL cephalothin in 50 mM phosphoric acid so as to have an appropriate concentration of cefamandole, mix, keep at 4° before injection, inject a 100 μL aliquot onto column A with mobile phase A,

elute column A with mobile phase A for 10 min, backflush the contents of column A onto column B with mobile phase B for 4 min, remove column A from the circuit, continue to elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 15 min.

HPLC VARIABLES

Column: A 40 × 2 37-50 μm Corasil RP C18; B 40 × 4.6 37-53 μm Co:Pell ODS + 250 × 4 10 μm LiChrosorb RP-8
Mobile phase: A 50 mM phosphoric acid; B MeOH:5 mM tetrabutylammonium bromide 45:55
Flow rate: A 1; B 1
Injection volume: 100
Detector: UV 270

CHROMATOGRAM

Retention time: 9 (cefamandole), 16 (cefamandole nafate)
Internal standard: cephalothin (14)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: cefotiam, cephalexin, cephaloridine, caffeine, cefotaxime, cefuroxime, cefazolin, cefoperazone, cefoxitin
Noninterfering: phenobarbital

KEY WORDS

plasma; column-switching

REFERENCE

Lee,H.S.; Zee,O.P.; Kwon,K.I. Simultaneous determination of cefamandole and cefamandole nafate in human plasma and urine by high-performance liquid chromatography with column switching, *J.Chromatogr.*, **1990**, *528*, 425–433.

SAMPLE

Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 100 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 20 (cefamandole), 48 (cefamandole nafate)
Limit of quantitation: 6400 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cephalothin

REFERENCE

Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE

Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 20:80
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 270

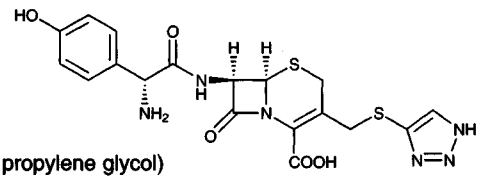
OTHER SUBSTANCES

Also analyzed: cephapirin

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Cefatrizine



Molecular formula: $C_{18}H_{18}N_6O_5S_2$
Molecular weight: 462.51
CAS Registry No.: 51627-14-6, 64217-62-5 (compd with propylene glycol)
Merck Index: 1965
Lednicer No.: 3 211

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.808

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.